Electronic Supplementary information

Carbon nano-dots as a fluorescent and colorimetric dual-readout probe for detection of arginine and Cu^{2+} and its logic gate operation

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Experimental

Apparatus

The UV−Vis absorption spectra were acquired on a PerkinElmer Lambda365 UV/Vis Spectrophotometer (PerkinElmer, USA). The fluorescence spectra were carried out on a Varian Cary Eclipse fluorescence spectrophotometer. A JEM-2100 electron microscope (Tokyo, Japan) operating at 300 kV was employed to obtain the transmission electron microscopic (TEM) and high resolution transmission electron microscopy (HRTEM) images. The Fourier transform infrared (FTIR) spectra were recorded on a Bruker Tensor Ⅱ FTIR spectrometer (Bremen, Germany). The crystal structure of CNDs was characterized by a Bruker D8 Advance X-ray diffractometer (λ=0.154056 nm). X-ray Photoelectron Spectroscopy (XPS) was recorded by a Shimadzu/Kratos AXIS ULTRA DLD photoelectron spectrometer (Kratos, Tokyo, Japan). The zeta potential was measured with a Malvern Zetasizer Nano-ZS90 dynamic light scattering system. Nanosecond fluorescence lifetime experiments were performed using a FLS 920 time-correlated single-photon counting (TCSPC) system under right-angle sample geometry. An Edinburgh EPL 405 nm picosecond diode laser with a repetition rate of 2 MHz (Livingston, UK) was used to excite the samples. The fluorescence was collected by a photomultiplier tube (Hamamatsu H5783p) connected to a Becker & Hickl SPC-130TCSPC board (Berlin, Germany).

Quantum yield measurement

The Φ_s of the three CDs were determined by a comparative method as follows:

$$\Phi_s = \Phi_R(\mathrm{Grad_S}/\mathrm{Grad_R})\ (\eta^2_S/\eta^2_R)$$

where Grad is the gradient from the plot of integrated fluorescence intensity against absorbance and η(1.33) is the refractive index of the solvent. The subscripts S and R represent CNDs and the reference (quinine sulfate in 0.10 M H_2SO_4). To prevent the re-absorption effect, the absorbances of three CNDs and quinine sulfate solutions in the 10mm fluorescence cuvette were kept under 0.10 at the excitation wavelength (λ_{ex}) of 365 nm. The integrated fluorescence intensity was the area under the PL curve in the wavelength range 380–680 nm. The Φ_R was taken as 0.54 since it is almost independent (within 5%) with λ_{ex} at 200–600 nm.[1]

Reference

1 X. Wang, K. Qu, B. Xu, J. Ren, X. Qu, *J. Mater. Chem.*, 2011, **21**, 2445-2450.

Cellular Toxicity Test

For the cell cytotoxicity test, human liver cancer HepG 2 cells were first plated on a Costar® 96-well cell culture cluster and cultured at 37℃ with 5.0% CO_2 in air for 3 h to adhere cells onto the surface. The well without cells and treatment with CNDs was taken as the zero sets. The medium was then changed with 200 μL of fresh DMEM supplemented with 10% FBS containing CNDs and the cells were allowed to grow for another 24h. At least six parallel samples were performed in each group. Cells not treated with CNDs were taken as the controls. After adding 20 μL of 5.0 mg mL^{-1} MTT reagent into every well, the cells were further incubated for 5 h. The culture medium with MTT was removed and 150 μL of DMSO was added. The resulting mixture was shaken for *ca.*10 min at room temperature. The optical density (OD) of the mixture was measured at 490 nm with a Sun Rise microplate reader (Tecan Austria GmbH, Grödig, Austria). The cell viability was estimated as: cell viability (%) = ($OD_{treated}$ /$OD_{control}$) × 100%, where $OD_{control}$ and $OD_{treated}$ were obtained in the absence and presence of CNDs, respectively.

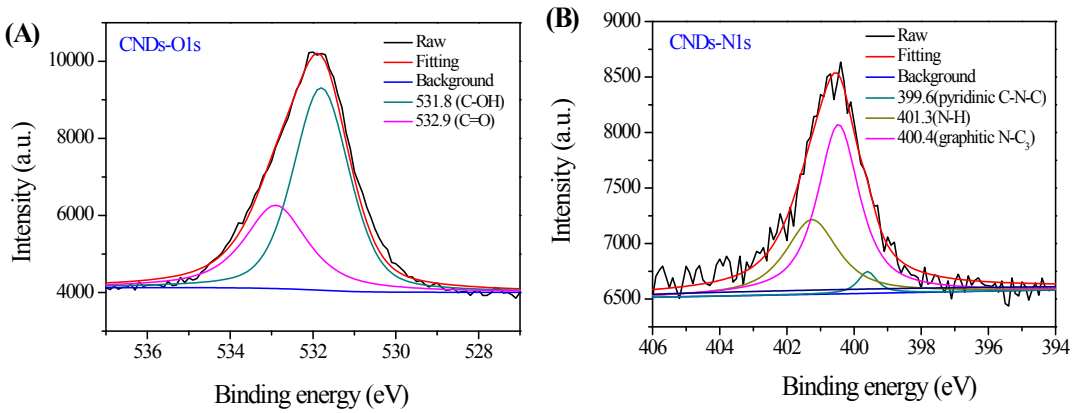

Fig. S1 High-resolution XPS data of O 1s (A) and N 1s (B) of CNDs.

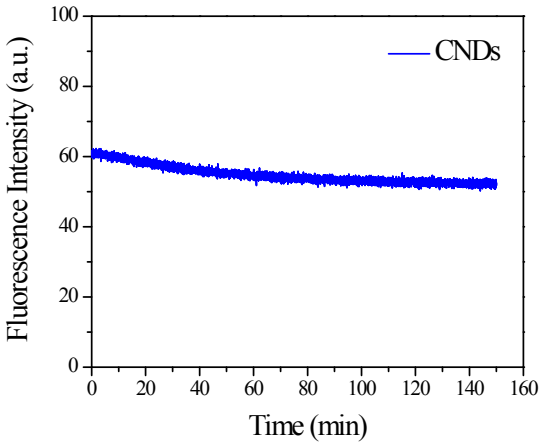

Fig. S2 Effect of time intervals of irradiation with xenon arc light on FL intensity of CNDs.

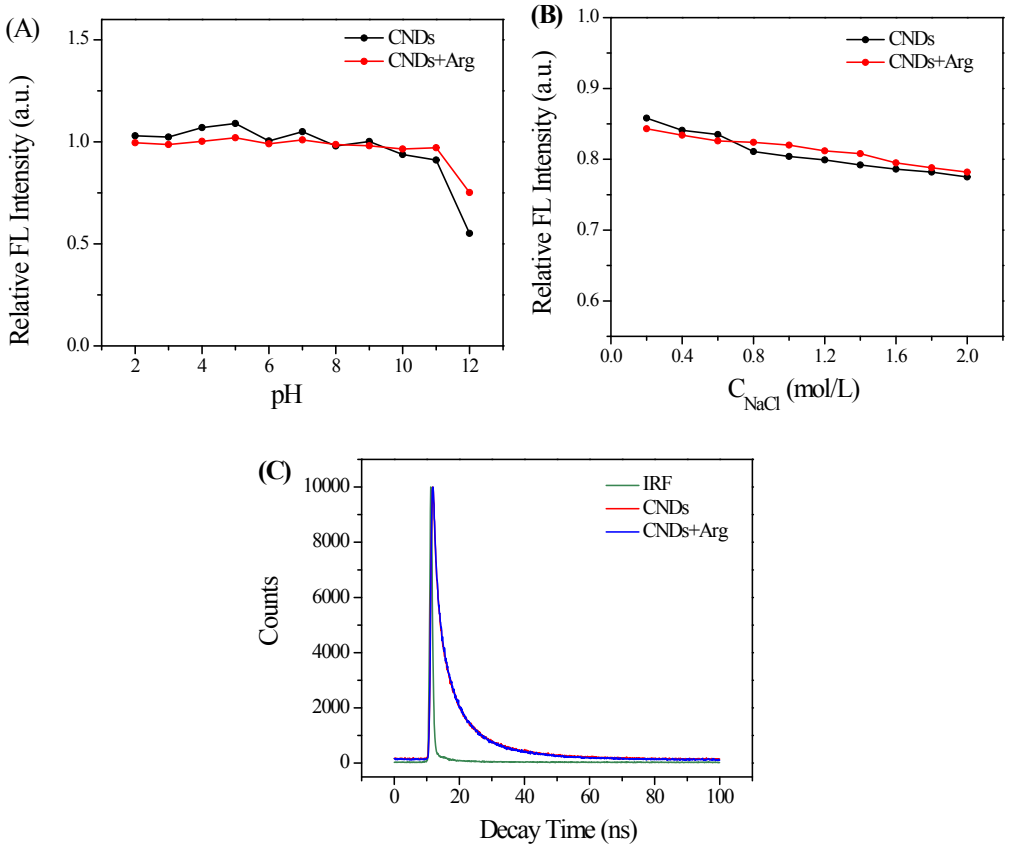

Fig. S3 (A) Effect of pH on FL intensity of CNDs and CND-Arg. The pH is adjusted by the PBS buffers. (B) Effect of ionic strength on FL intensity of CNDs and CND-Arg. The ionic strengths are controlled by various concentrations of NaCl. (C) Fluorescence lifetime of CNDs and CNDs-Arg, IRF is the instrumental response function curve. The concentration of CND and Arg are 0.50 mg mL^{-1} and 120 μM, respectively.

Table S1 Lifetime calculations from the time-resolved decay profiles of CNDs and CNDs-Arg.

Sample	τ_1 (ns)	Percentage (%)	τ_2 (ns)	Percentage (%)	Ave.τ (ns)
CNDs	4.0669	35.80	14.1924	64.20	10.57
CNDs-Arg	4.3020	38.41	14.3027	61.59	10.46

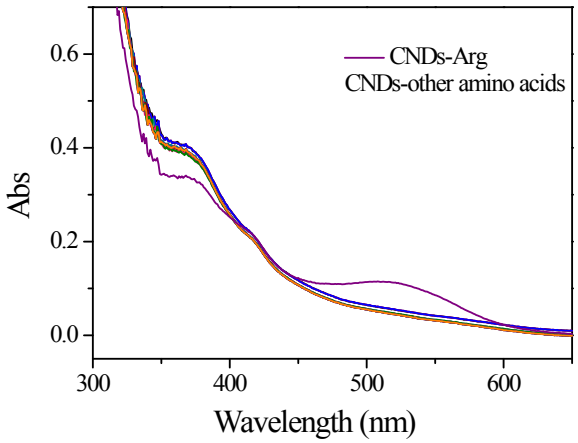

Fig. S4 UV-vis absorption spectra of the CNDs with various amino acids.

Table S2 Comparison of the detection limits of Arg from various analytical methods.

Sensing probe	**Method**	**Detection limits (μM)**	**Reference**
Rhodamine-thiourea/Al^{3+} complex	Fluorescence	2.30	2
Zn(II)-terpyridine complex	Fluorescence	2.05	3
Plumbagin	Fluorescence	1.0	4
DIlSD/SDS/Cu^{2+}	Fluorescence	0.17	5
Au/CQDs composite	UV–Vis	0.034	9
	Fluorescence	0.45	9
IPy/SDS/Cu^{2+}	Fluorescence	5.2×10^{-3}	23
Schiff base L	Fluorescence	0.67	25
$[PbL_2]^{2+}$ complex	Fluorescence	1.0×10^{-4}	25
Citrate-capped AuNPs	UV–Vis	0.016	62
CNDs	Fluorescence	0.26	This work*

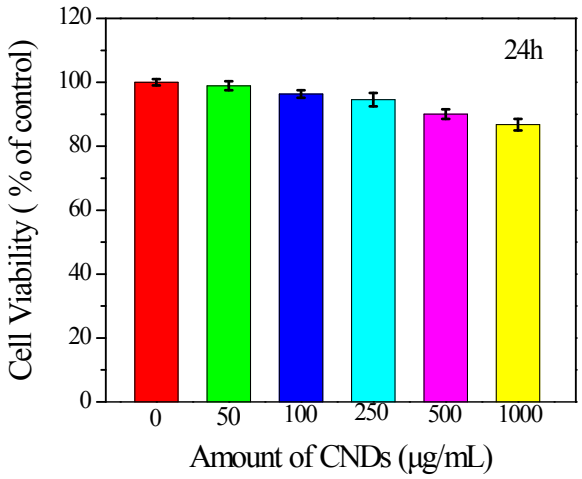

Fig. S5 Cytotoxicity testing results of CNDs on HepG 2 cells viability. The values represent percentage cell viability (mean% ± SD, n=6).